

Allelic variation in HMW glutenins in Spanish wheat landraces and their relationship with bread quality

P. Giraldo*, M. Rodríguez-Quijano, C. Simon,
J. F. Vazquez and J. M. Carrillo

Unidad de Genética. Departamento de Biotecnología. ETSI Agrónomos. Universidad Politécnica de Madrid.
Ciudad Universitaria. 28040 Madrid. Spain

Abstract

The allelic variation of high molecular weight glutenins as principal determinants of bread quality has been analyzed in 165 Spanish wheat (*Triticum aestivum* ssp. *vulgare* L.) landraces provided by the Plant Genetic Resources Centre. The identification by standard electrophoresis techniques has been supported by a new PCR screening method, allowing the identification of the 2•• glutenin subunit from the *Glu-A1* locus in some landraces. The relation of high molecular weight glutenins and bread quality has been evaluated by SDS-sedimentation tests and mixographs. A positive influence on quality has been found for the 2•• glutenin subunit from the *Glu-A1* locus, pairs 7 + 8 and 13 + 16 from the *Glu-B1* locus, and pair 5 + 10 from the *Glu-D1* locus. The presence of a wide range of values for quality traits in landraces with the same high molecular weight glutenin composition points to the possible influence of other prolamins such as the low molecular weight glutenins. Their influence on bread quality will be assessed in future studies. A complete description of the high molecular weight glutenin composition and quality values of all the landraces analyzed in this study is provided for use in wheat breeding programs.

Additional key words: mixograph; sedimentation volume; *Triticum aestivum*.

Resumen

Variación en gluteninas de alto peso molecular (HMW) en variedades tradicionales españolas de trigo y su relación con la calidad panadera

En este trabajo, se ha analizado la variación alélica para gluteninas de alto peso molecular (HMW) en una colección de 165 variedades tradicionales españolas de trigo blando (*Triticum aestivum* ssp. *vulgare* L.) provenientes del Centro Nacional de Recursos Fitogenéticos. La identificación mediante técnicas estándar de electroforesis se ha complementado con un método de análisis por PCR que ha permitido la identificación de la subunidad 2•• del locus *Glu-A1* en varios cultivares. La relación entre la composición en gluteninas de alto peso molecular y la calidad panadera se ha analizado mediante la prueba del volumen de sedimentación con SDS y los datos del mixógrafo. Se ha encontrado una influencia positiva en la calidad por parte de la subunidad 2•• del locus *Glu-A1*, los pares de subunidades 7 + 8 y 13 + 16 del locus *Glu-B1*, y el par 5 + 10 del locus *Glu-D1*. Variedades con la misma composición en gluteninas de alto peso molecular han mostrado un amplio rango de valores en los parámetros de calidad, apuntando a la participación de otras prolaminas, como las gluteninas de bajo peso molecular, cuya influencia en la calidad será analizada en el futuro. En el presente trabajo, se incluye la descripción detallada de la composición en gluteninas de alto peso molecular y de parámetros de calidad de todos los cultivares analizados para su posible utilización en programas de mejora de trigo.

Palabras clave adicionales: mixógrafo; *Triticum aestivum*; volumen de sedimentación.

* Corresponding author: patricia.giraldo@upm.es

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Abbreviations used: BDR (breakdown resistance), CRF-INIA (Centro de Recursos Fitogenéticos- Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria), HMW-GS (high molecular weight glutenin subunits), LMW-GS (low molecular weight glutenin subunits), MDT (mixograph development time), PCR (polymerase chain reaction), SDSS (sodium dodecyl sulphate sedimentation volume test).

Introduction

Many recent studies have looked at the high molecular weight glutenin subunit (HMW-GS) allelic variation among registered and old cultivars from different world regions. The lower commercial value of landraces (some of which are currently only cultivated in small regions of traditional farming and conserved in germplasm collections) has relegated them to second place. Nevertheless, wheat landraces are an important source of genetic variability for agronomic traits (resistance to abiotic and biotic stresses, adaptability, etc.) and they are essential in breeding programs to obtain new commercial cultivars of high quality and well adapted to different environments (Skovmand and Rajaram 1990). Moreover, landraces play an important role in low-input agriculture (Murphy *et al.*, 2005).

Bread quality depends on the viscoelastic properties of dough, and these are mainly determined by the quantity and quality of gluten proteins (Finney, 1943). Several studies have reported the relationship between the HMW-GS composition of wheat grain and the gluten strength of dough measured by different tests (sodium dodecyl sulphate sedimentation volume test, alveograph and mixograph) (Payne *et al.*, 1979, 1984, 1987; Pogna and Mellini, 1986; Branlard *et al.*, 1992). The allelic variation in HMW-GS has been reported to account for up to 60% of the variation in bread making quality (Payne *et al.*, 1987).

HMW-GS are encoded by the complex *Glu-1* loci located on the long arm of chromosomes from homeologous group 1 and called *Glu-A1*, *Glu-B1* and *Glu-D1* (Shewry *et al.*, 1992). In each chromosome, the locus contains two closely linked genes that encode for two polypeptides, x-type glutenin subunit and y-type glutenin subunit (Shewry *et al.*, 1992). Some of these genes are silent so most cultivars present from three to five different subunits. Thus, the *Glu-A1* locus encodes for zero or one glutenins (the y-type glutenin of this locus is not expressed) and the loci *Glu-B1* and *Glu-D1* encode for one or two glutenins each (Payne *et al.*, 1981).

The HMW-GS characterization in bread wheat breeding programs is essential to improve flour baking quality. They can be characterized easily by protein electrophoresis, however, in many cases it is difficult to distinguish small differences in the molecular weight of different glutenins. Fortunately, genome information is now available for the HMW-GS genes since most of the alleles have been cloned and sequenced,

allowing the development of molecular markers based on DNA sequence and PCR techniques (Liu *et al.*, 2008).

In modern cultivars, the HMW-GS allelic variability is not very large and the cultivars used for high strength wheat breeding are very similar in their HMW glutenin composition. Wheat landraces have a higher allelic variability and could be very useful for broadening the currently narrow genetic basis of modern cultivars in breeding programs (Christiansen *et al.*, 2005).

The bread-making quality of Spanish landraces has only been previously evaluated in a reduced set of 27 landraces by comparison with the quality of modern commercial cultivars (Gómez *et al.*, 2009). The relationship between quality and HMW-GS composition was not assessed in this study.

The overall objective of the present work was to characterize the HMW-GS composition and bread quality of a wide selection of Spanish wheat landraces (*Triticum aestivum* ssp. *vulgare* L.) conserved at the Plant Genetic Resources Centre (CRF-INIA) for their possible use in breeding programs.

Material and methods

We selected 165 bread wheat landraces from the Plant Genetic Resources Centre (CRF, INIA, Alcalá de Henares, Madrid, Spain, see appendix). Each landrace was sown in a single row (3 m long, 1.12 m apart) at the *ETSI Agrónomos* Agricultural Experimental Station (Madrid, Spain) in 2006–2007.

Isolation of genomic DNA from leaves was performed as previously described (Saghai-Marouf *et al.*, 1984). The primers G2F1 (5'-TTTCATACTATCCAGG CCAAGC-3') and G2R1 (5'-GGTTGTTGCCATTGT CCTGA-3') were designed with the Primer3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) from the sequences available in GenBank for the *Glu-A1x 2** and *Glu-A1x 2••* alleles (M22208 and DQ533690, respectively). Sequence alignment was performed with CLC Free Workbench (<http://www.clcbio.com>). PCR amplification was carried out in a 25 µL volume containing 100 ng of genomic DNA, 1x buffer (Biotools), 2 mM MgCl₂, 200 µM dNTPs, 0.5 µM primers and 0.75 U Taq DNA polymerase (Biotools). PCR was conducted in a MyCycler thermocycler (BioRad) as follows: heat denaturation at 94°C for 2 min, followed by 5 cycles of touchdown PCR (94°C for 30 s, an annealing step starting at 61°C for 1 min

and decreasing 1°C per cycle, and 72°C for 45 s), then 35 more cycles of PCR (94°C for 30 s, 56°C for 1 min, and 72°C for 45 s) and a final extension step 72°C for 5 min. Amplification products were analyzed in 3% NuSieve agarose gels (Cambrex) stained with GelRed (Biotium).

Protein extraction from the wholemeal flour was performed according to Singh *et al.* (1991). Extracted proteins were fractionated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) using 12% polyacrilamide gels as described by Payne *et al.* (1980).

Grain protein content (14% moisture basis) was measured by near-infrared reflectance analysis using the Technichon Infralyzer 300. The gluten strength was evaluated by SDS sedimentation volume test (SDSS) and mixograph. SDSS volume was measured according to Dick and Quick (1983). The mixograph variables were: mixing development time, MDT (time to maximum peak height in seconds), and breakdown resistance, BDR (difference in percentage between maximum peak height and height at 3 min after the peak). All measurements were run in duplicate using 10 g of flour in a National Manufacturing Co. Mixograph (Lincoln, NE), as described by Finney and Shogren (1972).

The statistical analysis of the data was performed with the SAS software (SAS Institute, Cary, NC, USA).

Results

Combining SDS-PAGE and PCR analysis we identified four different HMW-GS from the *Glu-A1* locus, nine different combinations from the *Glu-B1* locus and three different combinations from the *Glu-D1* locus in the 165 Spanish landraces analyzed. The 2* subunit and the 20x + 20y and 2 + 12 combinations were the most abundant in each locus (Table 1).

In the *Glu-A1* locus, the 1, 2* and Null glutenin subunits were easily identified by SDS-PAGE. This analysis showed the possible presence of the *Glu-A1x* 2•• glutenin subunit in some landraces. The correct assignment of this allele is difficult due to a similar electrophoretic mobility as the allelic 2* glutenin subunit. For unambiguous identification of the 2•• glutenin subunit, a screening method by PCR was designed. The alignment of 2•• and 2* nucleotide sequences showed a 18 bp deletion present in the 2* sequence and not in the 2•• sequence (Gobaa *et al.*, 2007). Two primers (G2F1 and G2R1) were designed to target this polymorphism. The G2F1 and G2R1 primers efficiently amplified a 100 bp fragment in the 2* glutenin subunit and a 118 bp fragment in the 2•• glutenin subunit of the *Glu-A1* locus (Fig. 1).

The gluten strength was evaluated by protein content, sedimentation test (SDSS) and two mixograph variables, MDT and BDR. The results are presented in Table 1.

Table 1. Mean values \pm standard deviation (SD) for protein content, SDSS sedimentation volume, mixograph variables MDT (mixograph development time) and BDR (breakdown resistance) for the 165 landraces analyzed. Samples are grouped by high molecular weight glutenin subunit (HMW-GS) composition in each *Glu-1* locus

| Locus | Allele | HMW-GS | N | % | Protein (%) Mean \pm SD | SDSS (mm) Mean \pm SD | MDT (s) Mean \pm SD | BDR (%) Mean \pm SD |
|---------------|--------|-----------|-----|------|------------------------------|----------------------------|--------------------------|--------------------------|
| <i>Glu-A1</i> | a | 1 | 22 | 13.3 | 14.1 \pm 0.8 | 63.6 \pm 19.5 | 50.7 \pm 27.4 | 42.0 \pm 6.1 |
| | b | 2* | 89 | 53.9 | 14.1 \pm 1.1 | 59.9 \pm 20.9 | 53.3 \pm 22.4 | 42.6 \pm 8.3 |
| | c | Null | 28 | 17.0 | 14.0 \pm 1.2 | 68.6 \pm 24.4 | 68.1 \pm 30.2 | 38.4 \pm 9.3 |
| | | 2•• | 26 | 15.8 | 13.3 \pm 0.9 | 72.2 \pm 11.2 | 71.8 \pm 26.7 | 37.8 \pm 7.1 |
| <i>Glu-B1</i> | a | 7 | 11 | 6.7 | 14.3 \pm 0.1 | 75.5 \pm 16.9 | 65.7 \pm 29.4 | 39.1 \pm 6.7 |
| | b | 7 + 8 | 7 | 4.2 | 13.4 \pm 0.7 | 84.6 \pm 9.7 | 82.4 \pm 17.4 | 32.1 \pm 7.3 |
| | d | 6 + 8 | 7 | 4.2 | 13.8 \pm 0.6 | 70.1 \pm 17.0 | 56.1 \pm 20.4 | 39.6 \pm 4.8 |
| | e | 20x + 20y | 110 | 66.7 | 14.0 \pm 1.1 | 57.5 \pm 20.1 | 50.0 \pm 20.1 | 43.3 \pm 8.2 |
| | f | 13 + 16 | 24 | 14.5 | 13.3 \pm 1.0 | 76.0 \pm 13.8 | 84.6 \pm 29.7 | 35.8 \pm 5.9 |
| | i | 17 + 18 | 1 | 0.6 | 16.8 \pm 0.4 | 100.5 \pm 2.0 | 114.0 \pm 3.2 | 35.4 \pm 0.7 |
| | as | 13 | 1 | 0.6 | 13.3 \pm 0.2 | 89.5 \pm 1.7 | 84.0 \pm 2.7 | 32.4 \pm 0.6 |
| | u | 7* + 8 | 2 | 1.2 | 15.6 \pm 1.1 | 56.8 \pm 8.8 | 31.0 \pm 7.3 | 44.7 \pm 5.3 |
| | | 7* + 9 | 2 | 1.2 | 14.1 \pm 1.2 | 83.0 \pm 19.8 | 75.0 \pm 15.6 | 30.9 \pm 1.2 |
| <i>Glu-D1</i> | a | 2 + 12 | 122 | 73.9 | 13.9 \pm 1.1 | 65.6 \pm 19.8 | 60.4 \pm 27.5 | 40.1 \pm 8.5 |
| | c | 4 + 12 | 40 | 24.2 | 13.9 \pm 1.1 | 56.6 \pm 21.1 | 50.4 \pm 20.4 | 41.2 \pm 7.6 |
| | d | 5 + 10 | 3 | 1.8 | 15.4 \pm 0.7 | 86.8 \pm 16.4 | 80.7 \pm 21.0 | 42.4 \pm 5.2 |

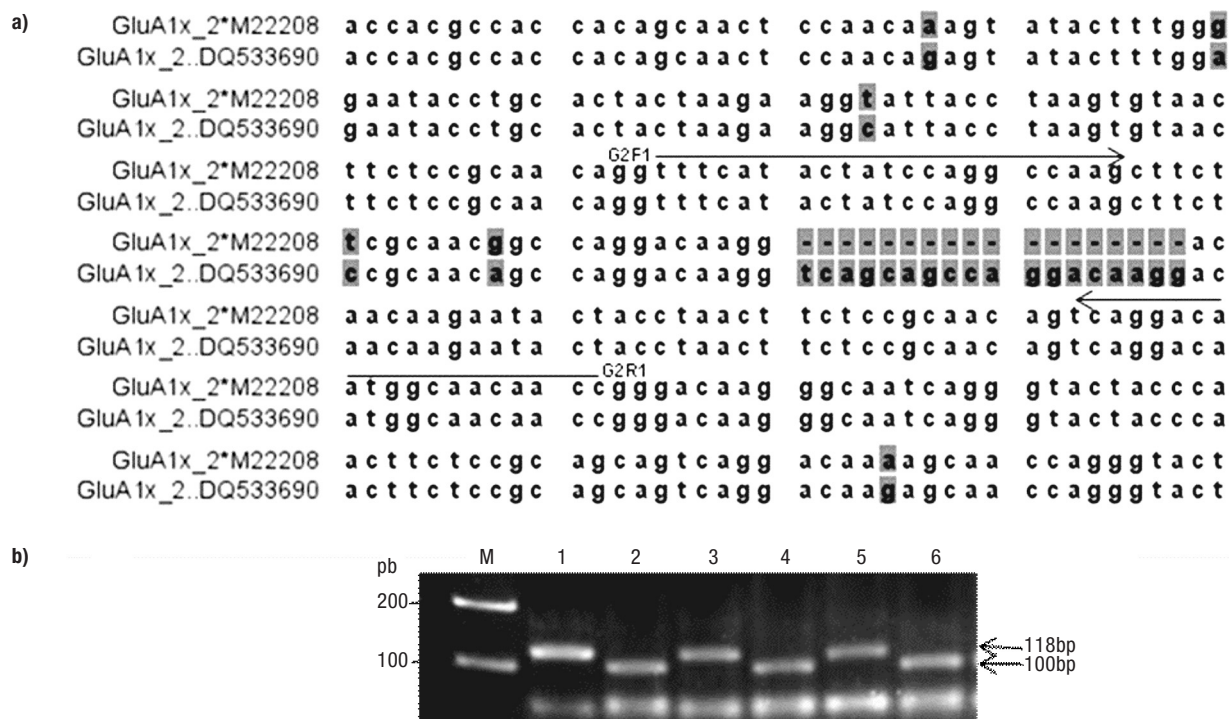


Figure 1. PCR screening for *GluA1x2*•• glutenin. a) Nucleotide alignment of a region of around 280 nucleotides from HMW glutenin subunit 1 (locus *Glu-A1*) glutenin gene coding for 2* and 2•• subunits. Alleles are labeled on the left side of sequences; the GeneBank accession number is also shown. This fragment corresponds to the region from nucleotides 2435 to 2714 of sequence M22208, and to the region from nucleotides 2638 to 2899 of sequence DQ533690. The polymorphism between both sequences is highlighted by the darker background and the 18 bp deletion is shown with dashes. b) PCR products amplified with G2F1 and G2R1 primers. Lanes 1, 3 and 5 are samples carrying the 2•• glutenin subunit; lanes 2, 4 and 6 are samples carrying the 2* glutenin subunit.

Mean protein content was around 14% in all the cases, with the widest ranges in the presence of the 7* + 8 pair of the *Gu-B1* locus and the 5 + 10 pair of the *Glu-D1* locus.

Landraces with the same HMW-GS composition showed a wide range of SDSS and mixograph values (Table 1, Fig. 2). The 2•• glutenin subunit had the highest mean SDSS value (72.2 mm) of all landraces with *Glu-A1* glutenins. In the case of the *Glu-B1* locus, the highest mean values were for the landraces carrying the 17 + 18, 13, and 7 + 8 pairs of glutenins (100.5, 89.5

and 84.6 mm, respectively) and the lowest value was for landraces with the 7* + 8 and 20x + 20y combination (56.8 and 57.5 mm, respectively). In the case of the *Glu-D1* locus, the highest and lowest mean SDSS values were for the 5 + 10 and 4 + 12 combinations respectively (Table 1).

The Null and 2•• subunits of the *Glu-A1* locus, the 17 + 18, 13, 13 + 16 and 7 + 8 pairs of *Glu-B1*, and the 5 + 10 pair of subunits of the *Glu-D1* locus had the highest mean values for the MDT and lowest for BDR (Table 1).

Pearson's correlation analysis showed a high and positive correlation between the SDSS volume and the MDT value ($R^2 = 0.82$; $p < 0.01$) and a high and negative correlation between the SDSS volume and the BDR value ($R^2 = -0.63$; $p < 0.01$). This means that a sample with a high sedimentation volume will also have a high mixing time value, which is a characteristic of dough with high stability, elasticity and mixing tolerance. Negative correlations with the BDR value will give a better resistance to over-kneading. Landraces with a high SDSS value also had a high MDT value and a low

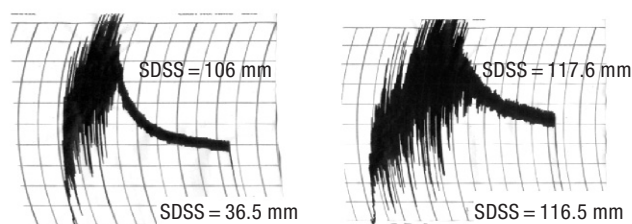


Figure 2. Mixograms of two landraces with the same high molecular weight glutenin composition (2*, 20x + 20y, 2 + 12). The SDS-Sedimentation test value is shown at the bottom.

BDR value, all of which are indicators of good dough development. Negative correlations were also found between the protein content and SDSS volume ($R^2 = -0.15$; $p < 0.05$) and MDT value ($R^2 = 0.36$; $p < 0.05$) and positive correlations were found between the protein content and the BDR value ($R^2 = 0.44$; $p < 0.01$).

The *t*-test statistical analysis for SDSS mean values according to HMW-GS composition is shown in Table 2. The 2•• glutenin subunit from the *Glu-A1* locus produced a high SDSS and low BDR value compared to 2* glutenin subunits, and higher mixograph values compared to Null and 1 subunits. The Null glutenin subunit produced higher mixograph values than 2* and 1 subunits. Similarly, the glutenin pairs 13 + 16 and 7 + 8 from the *Glu-B1* locus gave higher values than pairs 20x + 20y and 6 + 8. In the *Glu-D1* locus the 5 + 10 and 2 + 12 pairs gave higher SDSS and MDT

values than the 4 + 12 pair. Moreover, the MDT value of the 5 + 10 pair was significantly higher than that of the 2 + 12 pair.

Discussion

HMW-GS variation

In a previous study, for the *Glu-A1* locus in 308 Spanish landraces (53 of them also present in this work) Rodríguez-Quijano *et al.* (1990) found only the HMW-GS Null, 1, and 2* (with a frequency of 44%, 24% and 32% respectively); the same subunits that had been previously described by Payne and Lawrence (1983) in a set of 300 cultivars from all over the world. In a study of 1,380 landraces from Europe (Finland, France,

Table 2. Statistical *t*-test analysis of the influence on quality traits of high molecular weight glutenin subunit composition for each *Glu-1* locus

| Allelic composition compared | Trait ¹ | <i>t</i> -test ² |
|--|--------------------|-----------------------------|
| <i>Glu-A1</i> 2•• vs <i>Glu-A1</i> 1 | MDT | 2.7** |
| | BDR | -2.1* |
| <i>Glu-A1</i> 2•• vs <i>Glu-A1</i> 2* | SDSS | 2.9** |
| | MDT | 3.6** |
| | BDR | -2.6** |
| <i>Glu-A1</i> Null vs <i>Glu-A1</i> 2* | MDT | 2.9* |
| | BDR | -2.3* |
| <i>Glu-A1</i> Null vs <i>Glu-A1</i> 1 | MDT | 2.2* |
| <i>Glu-B1</i> 7 + 8 vs <i>Glu-B1</i> 20x + 20y | SDSS | 3.5** |
| | MDT | 4.1** |
| | BDR | -3.5** |
| <i>Glu-B1</i> 7 + 8 vs <i>Glu-B1</i> 6 + 8 | MDT | 2.6* |
| | BDR | -2.3** |
| <i>Glu-B1</i> 13 + 16 vs <i>Glu-B1</i> 20x + 20y | SDSS | 4.3** |
| | MDT | 6.9** |
| | BDR | -4.2** |
| <i>Glu-B1</i> 7 vs <i>Glu-B1</i> 20x + 20y | SDSS | 2.9* |
| | MDT | 2.4* |
| <i>Glu-B1</i> 13 + 16 vs <i>Glu-B1</i> 6 + 8 | MDT | 2.4* |
| <i>Glu-D1</i> 2 + 12 vs <i>Glu-D1</i> 4 + 12 | SDSS | 2.5* |
| | MDT | 2.1* |
| <i>Glu-D1</i> 5 + 10 vs <i>Glu-D1</i> 4 + 12 | SDSS | 2.4* |
| | MDT | 2.5* |
| <i>Glu-D1</i> 5 + 10 vs <i>Glu-D1</i> 2 + 12 | MDT | 1.3* |

¹ SDSS: SDS-Sedimentation volume. MDT: mixograph development time. BDR: breakdown resistance. ² *, **: $p < 0.05$ and $p < 0.01$, respectively.

Italy, UK), Asia (India, China, Pakistan), Australia and the Americas (Argentina, Brazil, Canada, México, USA) the same three HMW-GS were described with similar frequencies (33%, 32% and 36%) (Morgunov *et al.*, 1993). In four more recent works (Tohver, 2007; Singh *et al.*, 2007; Fang *et al.*, 2009; Xu *et al.*, 2009) with landraces from North and Central Europe, India and China, the allelic variation for the *Glu-A1* locus is also restricted to the Null, 1, and 2* glutenins. The absence of the 2•• glutenin subunit in all of these studies could be due to the difficult assignment of this glutenin subunit using SDS-PAGE electrophoresis, and some of the samples classified as 2* could, in fact, have been 2••. The 2•• glutenin subunit has been found, in the landraces analyzed in this work, at a similar frequency to 1 and Null glutenins. This glutenin subunit was first described in a work with 155 wheat lines derived from a Portuguese landrace called Barbela (Igrejas *et al.*, 1997). Subsequently, this glutenin subunit was described in a Portuguese cultivar called 'Ribeiro' (Brites *et al.*, 2000), in a North Spain *Triticum aestivum* ssp. *spelta* landrace (Elía, 2007) and in the breeding line '211.12014' from Switzerland (Gobaa *et al.*, 2007).

The development of a PCR screening method for the identification of this glutenin subunit has allowed easy and accurate identification. However, in the region amplified by the G2F1 and G2R1 primers there is no size polymorphism among 1, Null and 2•• glutenin subunits encoded by the *Glu-A1* locus, so, this method does not replace the SDS-PAGE analysis necessary for the other HMW glutenins. Additionally, some primers have been described to specifically detect the 2* subunit and discriminate it from Null and 1 glutenin subunits (De Bustos *et al.*, 2000; Liu *et al.*, 2008).

In the present work, the allelic variation for the *Glu-B1* locus is similar to that found in a previous study on Spanish landraces (Rodríguez-Quijano *et al.*, 1990). The high frequency of the pairs of subunits 20x + 20y and 13 + 16 is a strong difference from landraces from other world regions. In other previous works these two combinations have been found with a very low frequency, between 1 and 3% (Payne and Lawrence, 1983; Morgunov *et al.*, 1993; Tohver, 2007) or have not been found at all (Fang *et al.*, 2009; Li *et al.*, 2009). However, in studies with Portuguese landraces, the 20x + 20y and the 13 + 16 pairs have been found at a higher frequency: 50% and 17.2% (Rodríguez-Quijano *et al.*, 1990) and 5.8% and 23.1%, respectively (Brites *et al.*, 2000). The 13 + 16 pair has been also described at a high frequency (88%) in *Triticum aestivum* ssp. *spelta*

from North Spain (Rodríguez-Quijano *et al.*, 1990; Caballero *et al.*, 2004). These data confirm the conservation of these alleles in the Iberian Peninsula.

The HMW glutenins from the *Glu-D1* locus showed an allelic distribution similar to that found in other previous studies on Iberian landraces (Rodríguez-Quijano *et al.*, 1990; Brites *et al.*, 2000) and Chinese landraces, where the 2 + 12 pair was also the most frequent (Fang *et al.*, 2009; Li *et al.*, 2009) and different from the distribution found in studies on landraces from other world regions (Payne and Lawrence, 1983; Morgunov *et al.*, 1993; Tohver, 2007).

Quality analysis

Mean protein content was around 14% in all the landraces studied. Some authors (Branlard and Dardevet, 1985; Brunori *et al.*, 1989) have shown that dough strength is independent of this variable but the expression of a good quality genotype requires sufficient protein content. Although the protein content has no relationship with the HMW glutenin composition, it has some influence in mixograph variables. This influence has been analyzed in other studies, with contradictory results. Some authors have found a significant and positive correlation between SDSS values and protein content (Graybosch *et al.*, 1996), but others have not found any correlation (Dhaliwal *et al.*, 1987). We have found a significant, but low and negative, correlation between these variables, showing that, in general terms, wheat landraces with low protein content (12–13%) have a high SDSS value.

The 2•• glutenin subunit from the *Glu-A1* locus produced a high sedimentation volume compared to 1 and 2* glutenin subunits. The influence of the 2•• glutenin subunit on bread quality has been previously reported by Gobaa *et al.* (2007) who analyzed a population of double haploids. Nevertheless, this is the first time that its influence on quality has been analyzed in landraces.

To date, 1 and 2* glutenins subunits have been considered «good quality glutenins», both having a larger influence than the Null glutenin subunit (Cornish *et al.*, 2006). In this work, the mixograph values of landraces carrying the Null subunit have been significantly higher than the values of landraces with the 1 or 2* subunits. This discrepancy can be explained by the influence of HMW-GS from other loci. Landraces with the 1 or 2* glutenin subunits in the *Glu-A1* locus also

showed a high proportion of 20x + 20y glutenin subunits, a «bad quality» pair, in the *Glu-B1* locus. Conversely, in landraces with the Null glutenin subunit in the *Glu-A1* locus, the pair 20x + 20y is poorly represented.

The pairs of glutenins 13 + 16 and 7 + 8 from the *Glu-B1* locus have shown a high sedimentation volume compared to pairs 20x + 20y and 6 + 8. This result confirms previous studies that have considered both glutenin pairs of «bad quality» (Cornish *et al.*, 2006). The positive influence of the *Glu-D1* 5 + 10 pair compared to the 2 + 12 and 4 + 12 has also previously been shown (Cornish *et al.*, 2006). Most authors consider the 4 + 12 pair the one with the lowest influence on the sedimentation volume.

The large influence of HMW-GS on bread quality is widely known, but some influence of LMW-GS has also been reported (Payne *et al.*, 1987; Gupta *et al.*, 1989). This influence could explain the wide differences observed in quality traits (SDSS and mixograph) among landraces with the same HMW composition (Fig. 2). To address this issue, we have made a preliminary SDS-PAGE analysis and we have found that landraces with the same HMW glutenin subunit composition and different sedimentation volume also have different LMW composition, and landraces with similar LMW glutenins show similar sedimentation values (differences less than 15 mm, data not shown). To obtain an insight into the influence of the different LMW glutenins on bread quality we are generating advanced lines from crosses between landraces with the same HMW glutenin subunit composition and very different quality values.

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Appendix. List of the 165 bread wheat landraces (*Triticum aestivum* ssp. *vulgare* L.), provided by the Plant Genetic Resources Centre (CRF-INIA, Alcalá de Henares, Spain), analyzed in this study. The high molecular weight glutenin subunits (HMW-GS) composition, the sodium dodecyl sulphate sedimentation test value (SDSS), the grain protein content (Protein) and mixograph variables: peak mixing development time (MDT) and resistance to breakdown (BDR) are presented

| Genebank number | Local name | Region | HMW-GS | | | SDSS (mm) | Protein (%) | MDT (s) | BDR (%) |
|-----------------|--------------------------|-------------|---------------|---------------|---------------|-----------|-------------|---------|---------|
| | | | <i>Glu-A1</i> | <i>Glu-B1</i> | <i>Glu-D1</i> | | | | |
| BGE 1982 | Parrón | Asturias | 2* | 6 + 8 | 2 + 12 | 81.0 | 13.6 | 66 | 39.5 |
| BGE 2834 | Cañivano de Málaga | — | 2* | 20x + 20y | 2 + 12 | 65.0 | 15.6 | 48 | 46.3 |
| BGE 2836 | Caspino | — | Null | 20x + 20y | 2 + 12 | 34.5 | 14.6 | 38 | 49.4 |
| BGE 2838 | Catalán Rojo | — | 1 | 20x + 20y | 2 + 12 | 70.0 | 14.3 | 33 | 38.9 |
| BGE 2849 | Hembrilla de Jaca | Huesca | 1 | 20x + 20y | 2 + 12 | 52.0 | 12.3 | 33 | 36.4 |
| BGE 3540 | Trigo de Campo del País | Lugo | 2•• | 13 + 16 | 2 + 12 | 65.0 | 13.6 | 50 | 32.7 |
| BGE 3610 | Serodio | Lugo | 2* | 20x + 20y | 2 + 12 | 50.5 | 13.8 | 42 | 39.7 |
| BGE 3612 | Trigo Tremesino | Lugo | 2* | 20x + 20y | 2 + 12 | 74.5 | 14.7 | 45 | 40.5 |
| BGE 4789 | Trigo de Monte | Lugo | 2•• | 20x + 20y | 2 + 12 | 61.5 | 13.9 | 57 | 35.4 |
| BGE 5443 | Cascón | Palencia | Null | 7 | 2 + 12 | 110.0 | 12.4 | 144 | 21.0 |
| BGE 8707 | Barbilla de Sevilla | — | 2* | 20x + 20y | 5 + 10 | 84.0 | 16.2 | 80 | 38.5 |
| BGE 10003 | Caña Gruesa | Palencia | 2* | 7* + 9 | 2 + 12 | 97.0 | 14.9 | 96 | 31.2 |
| BGE 11826 | Perdigón | Cáceres | 2•• | 13 + 16 | 2 + 12 | 60.0 | 13.7 | 45 | 38.5 |
| BGE 11869 | Blanco de Segarra | Lérida | 2* | 20x + 20y | 2 + 12 | 64.5 | 13.3 | 50 | 34.4 |
| BGE 11870 | Chamorro de Hellín | Albacete | 2* | 7* + 9 | 2 + 12 | 69.0 | 13.2 | 54 | 30.7 |
| BGE 11872 | Nápoles de Alcaraz | Albacete | 2•• | 13 + 16 | 2 + 12 | 57.5 | 12.9 | 72 | 36.6 |
| BGE 11874 | Jeja Blanca | Tarragona | 2* | 20x + 20y | 2 + 12 | 55.5 | 13.8 | 60 | 42.9 |
| BGE 11887 | Escandín de Salas | Asturias | Null | 7* + 8 | 2 + 12 | 50.5 | 14.8 | 30 | 38.6 |
| BGE 11935 | Portugués | Cáceres | 2•• | 7 | 2 + 12 | 73.5 | 14.4 | 50 | 34.8 |
| BGE 11947 | Jeja Mocha | Barcelona | 2* | 20x + 20y | 2 + 12 | 54.5 | 14.9 | 57 | 44.2 |
| BGE 11950 | Cabezorro | Cáceres | 2•• | 13 + 16 | 2 + 12 | 86.5 | 12.8 | 69 | 41.5 |
| BGE 11955 | Chamorro de Viveros | Albacete | 2* | 20x + 20y | 4 + 12 | 59.0 | 12.9 | 32 | 32.2 |
| BGE 11959 | Mocho Velloso de Garay | Vizcaya | 2* | 20x + 20y | 5 + 10 | 72.0 | 14.8 | 60 | 48.4 |
| BGE 11985 | Montnegre | Barcelona | Null | 7 + 8 | 2 + 12 | 84.0 | 13.9 | 86 | 40.0 |
| BGE 11995 | Negrete de Priego | Cuenca | 2* | 20x + 20y | 2 + 12 | 57.0 | 13.3 | 33 | 33.9 |
| BGE 11998 | Cerrudo | Huesca | Null | 6 + 8 | 2 + 12 | 83.0 | 12.9 | 60 | 38.7 |
| BGE 11999 | Cerrudo | Huesca | 2* | 20x + 20y | 4 + 12 | 35.0 | 12.5 | 46 | 36.4 |
| BGE 12011 | Miranda | Asturias | 2* | 20x + 20y | 4 + 12 | 69.5 | 12.8 | 42 | 34.5 |
| BGE 12012 | Somiedo | Asturias | 2* | 6 + 8 | 2 + 12 | 92.0 | 13.9 | 60 | 38.8 |
| BGE 12019 | Negrete de Cuenca | Cuenca | 2* | 20x + 20y | 4 + 12 | 45.5 | 13.3 | 42 | 48.5 |
| BGE 12025 | Negrete de Priego | Cuenca | 2* | 20x + 20y | 4 + 12 | 25.0 | 13.5 | 48 | 45.4 |
| BGE 12039 | Negrillo de Alcolea | Guadalajara | 2* | 20x + 20y | 2 + 12 | 48.0 | 13.9 | 12 | 42.1 |
| BGE 12048 | Blanquillo de Bonillo | Albacete | 2* | 20x + 20y | 4 + 12 | 60.5 | 15.1 | 33 | 48.7 |
| BGE 12056 | Negrete de Altarejos | Cuenca | 2* | 20x + 20y | 4 + 12 | 35.5 | 12.9 | 24 | 41.1 |
| BGE 12061 | Negrete de Cuenca | Cuenca | 2* | 20x + 20y | 4 + 12 | 30.5 | 13.1 | 37 | 46.1 |
| BGE 12067 | Blanquillo | Soria | 2* | 20x + 20y | 4 + 12 | 25.0 | 12.6 | 38 | 43.5 |
| BGE 12083 | Negrete de Huelves | Cuenca | 2* | 20x + 20y | 4 + 12 | 53.5 | 13.8 | 30 | 47.3 |
| BGE 12092 | Negro de Salas | Asturias | 2* | 20x + 20y | 4 + 12 | 41.0 | 12.8 | 33 | 40.3 |
| BGE 12112 | Blanco Mocho | León | 2•• | 20x + 20y | 4 + 12 | 73.0 | 12.5 | 48 | 36.1 |
| BGE 12114 | Pelado | Cáceres | 2•• | 20x + 20y | 4 + 12 | 79.5 | 12.9 | 60 | 45.7 |
| BGE 12122 | Xexa Candéal | Baleares | 2* | 20x + 20y | 2 + 12 | 74.0 | 13.0 | 42 | 38.8 |
| BGE 12124 | Richela Blanca | Lérida | 2* | 20x + 20y | 2 + 12 | 95.0 | 12.8 | 83 | 93.4 |
| BGE 12125 | Mocho | Zamora | Null | 7 + 8 | 4 + 12 | 85.5 | 12.5 | 96 | 21.2 |
| BGE 12127 | Candéal de Sierra Nevada | Granada | 2* | 20x + 20y | 2 + 12 | 67.5 | 13.1 | 42 | 40.3 |
| BGE 12128 | Chamorro de Humanes | Guadalajara | 2* | 20x + 20y | 2 + 12 | 81.0 | 13.2 | 87 | 39.9 |
| BGE 12129 | Chamorro de Madrigueras | Albacete | 2* | 7 | 2 + 12 | 81.0 | 13.1 | 87 | 39.0 |
| BGE 12130 | Mocho Blanco | Salamanca | Null | 13 + 16 | 2 + 12 | 76.0 | 14.5 | 48 | 42.9 |
| BGE 12193 | Mocho de las Regueras | Asturias | 2* | 20x + 20y | 2 + 12 | 53.5 | 13.2 | 33 | 47.5 |
| BGE 12194 | Hernani | Guipuzcoa | 2* | 20x + 20y | 4 + 12 | 54.0 | 14.5 | 30 | 50.0 |

Appendix (cont.). List of the 165 bread wheat landraces (*Triticum aestivum* ssp. *vulgare* L.), provided by the Plant Genetic Resources Centre (CRF-INIA, Alcalá de Henares, Spain), analyzed in this study. The high molecular weight glutenin subunits (HMW-GS) composition, the sodium dodecyl sulphate sedimentation test value (SDSS), the grain protein content (Protein) and mixograph variables: peak mixing development time (MDT) and resistance to breakdown (BDR) are presented

| Genebank number | Local name | Region | HMW-GS | | | SDSS (mm) | Protein (%) | MDT (s) | BDR (%) |
|-----------------|-----------------------------|-------------|---------------|---------------|---------------|-----------|-------------|---------|---------|
| | | | <i>Glu-A1</i> | <i>Glu-B1</i> | <i>Glu-D1</i> | | | | |
| BGE 12205 | Chamorro de Villadiego | Burgos | Null | 20x + 20y | 2 + 12 | 108.0 | 14.1 | 89 | 28.1 |
| BGE 12206 | Toseta de Soria | Soria | 2* | 20x + 20y | 2 + 12 | 84.5 | 14.4 | 57 | 40.0 |
| BGE 12210 | Mocho Rojo | Ávila | Null | 7 + 8 | 2 + 12 | 102.0 | 13.8 | 105 | 25.1 |
| BGE 12211 | Blanquillo de Matamorosa | Cantabria | Null | 7 + 8 | 4 + 12 | 90.0 | 14.3 | 94 | 27.8 |
| BGE 12212 | Mocho de Arróniz | Navarra | 2* | 20x + 20y | 4 + 12 | 60.0 | 14.1 | 54 | 37.3 |
| BGE 12214 | Mocho Rioja | Álava | 2* | 20x + 20y | 4 + 12 | 65.5 | 13.5 | 45 | 45.1 |
| BGE 12238 | Involcable | Álava | 2* | 7 | 2 + 12 | 95.5 | 15.2 | 75 | 39.1 |
| BGE 12239 | Richela Blanca | Lérida | 2* | 17 + 18 | 2 + 12 | 100.5 | 16.8 | 114 | 35.3 |
| BGE 12575 | Candeal de Muñogrande | Ávila | Null | 20x + 20y | 2 + 12 | 61.0 | 13.4 | 60 | 39.7 |
| BGE 12576 | Candeal Fino de Minaya | Albacete | 1 | 20x + 20y | 4 + 12 | 65.5 | 13.5 | 42 | 41.5 |
| BGE 12577 | Candeal de Arévalo | — | 2* | 20x + 20y | 2 + 12 | 92.0 | 13.3 | 99 | 33.0 |
| BGE 12580 | Pichi | Badajoz | 2* | 20x + 20y | 5 + 10 | 104.5 | 15.1 | 102 | 40.2 |
| BGE 12582 | Blanquillo de Toledo | Toledo | 2•• | 13 + 16 | 2 + 12 | 68.5 | 12.3 | 72 | 44.9 |
| BGE 12586 | Candeal de Alcalá | Madrid | Null | 20x + 20y | 2 + 12 | 70.5 | 13.4 | 60 | 38.5 |
| BGE 12591 | Candeal de Vellisca | Cuenca | 2* | 20x + 20y | 2 + 12 | 116.5 | 14.2 | 128 | 33.3 |
| BGE 12595 | Gabatx | Gerona | 2* | 20x + 20y | 2 + 12 | 52.5 | 13.5 | 51 | 42.8 |
| BGE 12596 | Hembrilla de Alfaro | La Rioja | 2* | 20x + 20y | 2 + 12 | 70.0 | 12.8 | 45 | 46.0 |
| BGE 12596 | Rojo de Campos | Palencia | 2•• | 20x + 20y | 4 + 12 | 90.5 | 12.7 | 86 | 36.2 |
| BGE 12603 | Caravaca | Murcia | 1 | 20x + 20y | 2 + 12 | 62.5 | 14.3 | 45 | 41.0 |
| BGE 12207 | Toseta de Jaca | Huesca | 2* | 20x + 20y | 2 + 12 | 72.0 | 14.6 | 60 | 45.5 |
| BGE 12744 | Xexa de Mar | Barcelona | 1 | 6 + 8 | 2 + 12 | 57.5 | 14.2 | 39 | 39.4 |
| BGE 12746 | Rojo de Eslava | Navarra | 1 | 20x + 20y | 2 + 12 | 83.0 | 13.9 | 53 | 43.7 |
| BGE 12752 | Negro | Madrid | 2* | 20x + 20y | 2 + 12 | 60.5 | 16.2 | 27 | 42.3 |
| BGE 12761 | Catalán de Monte | Zaragoza | 2* | 20x + 20y | 4 + 12 | 38.5 | 15.7 | 60 | 29.6 |
| BGE 12785 | Jeja Manchega | — | 1 | 6 + 8 | 2 + 12 | 52.5 | 14.0 | 33 | 33.9 |
| BGE 12861 | Jeja Manchega | Ciudad Real | 2•• | 20x + 20y | 4 + 12 | 66.0 | 14.40 | 63 | 49.5 |
| BGE 12875 | Trigo del País | Asturias | 1 | 6 + 8 | 2 + 12 | 76.0 | 13.50 | 93 | 38.5 |
| BGE 12888 | Trigo de Riego | Pontevedra | 2•• | 13 + 16 | 2 + 12 | 71.0 | 12.80 | 81 | 35.8 |
| BGE 12889 | Trigo del País | Cantabria | 2* | 20x + 20y | 2 + 12 | 73.0 | 14.8 | 45 | 37.3 |
| BGE 12893 | Trigo de Riego | Pontevedra | 2* | 20x + 20y | 4 + 12 | 45.0 | 15.4 | 33 | 46.1 |
| BGE 12894 | Rojo del País | Lérida | Null | 13 + 16 | 2 + 12 | 70.0 | 11.7 | 93 | 37.2 |
| BGE 12896 | Alcaraz Negro | Albacete | Null | 7* + 8 | 2 + 12 | 63.0 | 16.3 | 42 | 50.9 |
| BGE 13123 | Blanco Entrefino de Oropesa | Toledo | 2* | 20x + 20y | 2 + 12 | 62.5 | 16.2 | 57 | 42.6 |
| BGE 13125 | Hembrilla de Rueda | Valladolid | 2* | 20x + 20y | 2 + 12 | 65.0 | 13.0 | 39 | 52.0 |
| BGE 13128 | Candeal de Tierra de Campos | Salamanca | 2•• | 20x + 20y | 2 + 12 | 69.5 | 13.5 | 48 | 42.7 |
| BGE 13131 | Candeal de Salamanca | Salamanca | 2•• | 13 + 16 | 2 + 12 | 68.5 | 13.0 | 57 | 33.3 |
| BGE 13133 | Candeal de La Sagra | Toledo | 2•• | 20x + 20y | 2 + 12 | 80.0 | 12.3 | 90 | 31.4 |
| BGE 13134 | Candeal de Aranda | Burgos | Null | 20x + 20y | 2 + 12 | 65.5 | 13.1 | 57 | 39.5 |
| BGE 13137 | Mahón | Baleares | 2* | 20x + 20y | 2 + 12 | 71.0 | 14.6 | 69 | 38.2 |
| BGE 13149 | Blanquillo | Toledo | 2•• | 13 + 16 | 2 + 12 | 80.5 | 13.8 | 129 | 28.0 |
| BGE 13151 | Trigo del País | Orense | 2•• | 13 + 16 | 2 + 12 | 87.0 | 14.7 | 123 | 24.0 |
| BGE 13152 | Canet | Gerona | 1 | 20x + 20y | 2 + 12 | 43.0 | 15.2 | 17 | 45.8 |
| BGE 13153 | Jeja Colorada de Albacete | Albacete | 1 | 20x + 20y | 2 + 12 | 61.0 | 13.6 | 54 | 47.0 |
| BGE 13155 | Tremesino | Cáceres | Null | 20x + 20y | 2 + 12 | 65.0 | 14.3 | 66 | 35.1 |
| BGE 13158 | Tremesino de Gredos | Ávila | 2* | 20x + 20y | 2 + 12 | 78.5 | 12.7 | 78 | 36.2 |
| BGE 13162 | Gandal | Lugo | 2•• | 13 + 16 | 2 + 12 | 74.0 | 13.8 | 51 | 40.0 |
| BGE 13164 | Jeja Mallorquina | Baleares | 2* | 20x + 20y | 2 + 12 | 81.0 | 14.7 | 75 | 37.5 |
| BGE 13166 | Menudillo | Zaragoza | Null | 20x + 20y | 2 + 12 | 35.5 | 16.3 | 33 | 56.8 |
| BGE 13179 | Colorado de Alfaro | La Rioja | Null | 13 + 16 | 2 + 12 | 71.0 | 14.0 | 94 | 31.9 |

Appendix (cont.). List of the 165 bread wheat landraces (*Triticum aestivum* ssp. *vulgare* L.), provided by the Plant Genetic Resources Centre (CRF-INIA, Alcalá de Henares, Spain), analyzed in this study. The high molecular weight glutenin subunits (HMW-GS) composition, the sodium dodecyl sulphate sedimentation test value (SDSS), the grain protein content (Protein) and mixograph variables: peak mixing development time (MDT) and resistance to breakdown (BDR) are presented

| Genebank number | Local name | Region | HMW-GS | | | SDSS (mm) | Protein (%) | MDT (s) | BDR (%) |
|-----------------|--------------------------------|-------------|---------------|---------------|---------------|-----------|-------------|---------|---------|
| | | | <i>Glu-A1</i> | <i>Glu-B1</i> | <i>Glu-D1</i> | | | | |
| BGE 13181 | Jeja Cañifina | Tarragona | 2* | 20x+20y | 4+12 | 73.5 | 13.7 | 53 | 43.5 |
| BGE 13182 | Jeja | Castellón | 2* | 20x+20y | 4+12 | 75.0 | 13.4 | 57 | 39.0 |
| BGE 13183 | Blanquillo de Albacete | Albacete | 1 | 20x+20y | 2+12 | 62.0 | 14.4 | 42 | 49.3 |
| BGE 13184 | Corriente de Jaca | Huesca | 2* | 20x+20y | 2+12 | 40.5 | 15.3 | 30 | 47.7 |
| BGE 13185 | Valle de Arán | Lérida | 2* | 20x+20y | 4+12 | 52.5 | 14.0 | 44 | 46.2 |
| BGE 13186 | Bergantiños | La Coruña | 2•• | 13+16 | 2+12 | 92.0 | 11.8 | 112 | 34.4 |
| BGE 13192 | Manzanares | Ciudad Real | 2* | 20x+20y | 2+12 | 41.0 | 13.9 | 45 | 49.1 |
| BGE 13195 | Barbilla de Carbajales de Alba | Zamora | 2•• | 13+16 | 2+12 | 81.5 | 12.1 | 135 | 21.7 |
| BGE 13197 | Rojo de Paredes | Cuenca | 2* | 20x+20y | 2+12 | 48.5 | 15.3 | 33 | 55.9 |
| BGE 13198 | Valenciano | Murcia | 2* | 20x+20y | 2+12 | 44.0 | 14.3 | 30 | 48.2 |
| BGE 13199 | Tremesino de Olivenza | Badajoz | 1 | 20x+20y | 2+12 | 38.5 | 14.0 | 21 | 46.4 |
| BGE 13203 | Cañamaciza | Palencia | 2* | 20x+20y | 2+12 | 32.0 | 14.9 | 27 | 48.2 |
| BGE 13216 | Santa Marta | Badajoz | 1 | 7 | 2+12 | 78.8 | 14.5 | 57 | 43.5 |
| BGE 13218 | Labradío | La Coruña | Null | 13+16 | 2+12 | 96.5 | 14.4 | 126 | 36.5 |
| BGE 13760 | Isla de Fuerteventura | Las Palmas | Null | 13+16 | 2+12 | 74.0 | 14.3 | 80 | 38.8 |
| BGE 13762 | Blanquillo de Cáceres | Cáceres | 2* | 13+16 | 2+12 | 62.0 | 12.7 | 77 | 34.9 |
| BGE 13764 | Gabatx de Fornes | Gerona | 2•• | 20x+20y | 4+12 | 81.5 | 13.5 | 73 | 35.0 |
| BGE 13767 | Montjuich | Barcelona | Null | 20x+20y | 2+12 | 38.5 | 16.6 | 27 | 61.7 |
| BGE 13769 | Casanya de Olot | Gerona | 2* | 20x+20y | 2+12 | 41.0 | 15.8 | 36 | 50.0 |
| BGE 13771 | Pirineos | Huesca | 2•• | 20x+20y | 2+12 | 46.5 | 15.4 | 39 | 51.5 |
| BGE 13773 | Montearagón | Teruel | Null | 20x+20y | 2+12 | 37.5 | 12.7 | 45 | 42.2 |
| BGE 13776 | Monte | Cantabria | 1 | 20x+20y | 2+12 | 47.5 | 14.0 | 57 | 40.0 |
| BGE 13795 | Gamonal | Toledo | 2* | 13 | 2+12 | 89.5 | 13.3 | 84 | 32.4 |
| BGE 13798 | Mentana | Soria | 1 | 7 | 2+12 | 69.0 | 14.3 | 66 | 41.0 |
| BGE 13800 | Hembrilla de Bleuca | Huesca | 1 | 6+8 | 2+12 | 48.5 | 14.8 | 42 | 48.8 |
| BGE 13802 | Vigo Cañifino | Pontevedra | 2•• | 13+16 | 2+12 | 68.5 | 12.3 | 66 | 40.6 |
| BGE 13811 | Royo de Pamplona | Navarra | 2* | 20x+20y | 4+12 | 63.0 | 14.7 | 69 | 45.7 |
| BGE 14261 | Vimbodi | Tarragona | 2* | 20x+20y | 2+12 | 78.5 | 13.4 | 78 | 40.2 |
| BGE 14281 | Xexa de Alicante | Alicante | 2* | 20x+20y | 2+12 | 49.0 | 13.3 | 63 | 35.6 |
| BGE 14285 | Candeal de Vellisca | Cuenca | 1 | 20x+20y | 4+12 | 82.3 | 13.8 | 76 | 36.6 |
| BGE 15396 | Jeja Colorada | Cuenca | 2* | 20x+20y | 2+12 | 34.5 | 12.7 | 30 | 51.3 |
| BGE 15404 | Trigo de Aruga | Cáceres | 2•• | 13+16 | 2+12 | 55.5 | 13.3 | 81 | 43.2 |
| BGE 18195 | Candeal fino de Minaya | Albacete | 2* | 20x+20y | 2+12 | 69.0 | 13.6 | 65 | 41.0 |
| BGE 18196 | Jeja Candeal | Valencia | 2* | 20x+20y | 2+12 | 50.0 | 15.7 | 51 | 42.2 |
| BGE 18200 | Bonito | Cáceres | 2•• | 13+16 | 2+12 | 75.5 | 13.4 | 57 | 42.8 |
| BGE 18202 | Jeja Pardilla | Cuenca | 2* | 20x+20y | 2+12 | 41.0 | 14.0 | 30 | 47.9 |
| BGE 18207 | Rojo de Caravaca | Murcia | 2* | 20x+20y | 2+12 | 36.0 | 13.6 | 43 | 41.9 |
| BGE 18208 | Coaña Blanco | Asturias | Null | 13+16 | 2+12 | 77.5 | 14.6 | 77 | 35.4 |
| BGE 18211 | Lebaniego | Palencia | 2* | 20x+20y | 2+12 | 53.5 | 12.3 | 39 | 46.1 |
| BGE 18214 | Rojo de Villaseca | Toledo | Null | 20x+20y | 4+12 | 14.5 | 14.5 | 30 | 39.5 |
| BGE 18215 | Rendía | Murcia | Null | 7+8 | 2+12 | 71.0 | 13.6 | 73 | 36.6 |
| BGE 18217 | Rojo de Humanes | Guadalajara | Null | 7+8 | 4+12 | 81.5 | 13.3 | 63 | 38.2 |
| BGE 18219 | Almendral Rojo Oscuro | Badajoz | 2* | 7 | 2+12 | 67.0 | 15.2 | 60 | 39.9 |
| BGE 18222 | Jeja de Cieza | Murcia | 2* | 20x+20y | 2+12 | 54.0 | 14.2 | 46 | 51.7 |
| BGE 18225 | Colorado de Soria | Soria | 2* | 7 | 2+12 | 44.5 | 14.3 | 46 | 43.0 |
| BGE 18226 | Colorado | Tenerife | 1 | 7 | 2+12 | 70.0 | 14.5 | 51 | 43.4 |
| BGE 18227 | Jeja de Puerto Lápite | Ciudad Real | 2* | 20x+20y | 2+12 | 27.0 | 13.0 | 60 | 37.9 |
| BGE 18228 | Barbilla de León | León | 1 | 13+16 | 2+12 | 117.5 | 12.0 | 146 | 27.8 |
| BGE 18229 | Cangrejero | Cangrejero | 2* | 20x+20y | 2+12 | 32.5 | 11.9 | 48 | 39.0 |

Appendix (cont.). List of the 165 bread wheat landraces (*Triticum aestivum* ssp. *vulgare* L.), provided by the Plant Genetic Resources Centre (CRF-INIA, Alcalá de Henares, Spain), analyzed in this study. The high molecular weight glutenin subunits (HMW-GS) composition, the sodium dodecyl sulphate sedimentation test value (SDSS), the grain protein content (Protein) and mixograph variables: peak mixing development time (MDT) and resistance to breakdown (BDR) are presented

| Genebank number | Local name | Region | HMW-GS | | | SDSS (mm) | Protein (%) | MDT (s) | BDR (%) |
|-----------------|-------------------------|-----------|---------------|---------------|---------------|-----------|-------------|---------|---------|
| | | | <i>Glu-A1</i> | <i>Glu-B1</i> | <i>Glu-D1</i> | | | | |
| BGE 18230 | Hembrilla de Soria | Soria | 2* | 20x + 20y | 4 + 12 | 77.5 | 13.5 | 90 | 40.0 |
| BGE 18231 | Grosal | Teruel | 2* | 20x + 20y | 4 + 12 | 70.0 | 13.5 | 84 | 25.3 |
| BGE 18233 | Vergara Temprano | Guipuzcoa | 1 | 7 | 2 + 12 | 76.3 | 15.2 | 45 | 46.3 |
| BGE 18234 | Barbilla Roja | Huelva | 2* | 13 + 16 | 2 + 12 | 87.0 | 15.2 | 90 | 35.2 |
| BGE 18239 | Castrojeriz | Burgos | 2* | 20x + 20y | 4 + 12 | 40.0 | 15.9 | 30 | 50.0 |
| BGE 18242 | Xeixa | Lérida | 2* | 20x + 20y | 4 + 12 | 31.5 | 15.0 | 30 | 51.9 |
| BGE 18244 | Cabeza Negra | Huesca | 2* | 20x + 20y | 4 + 12 | 38.5 | 15.4 | 33 | 51.0 |
| BGE 18248 | Jeja Fina | Teruel | 2* | 20x + 20y | 4 + 12 | 21.5 | 17.2 | 22 | 55.7 |
| BGE 18249 | Blat Petit de Olot | Gerona | Null | 20x + 20y | 2 + 12 | 26.0 | 15.5 | 45 | 47.9 |
| BGE 18253 | Radondell de Las Llosas | Gerona | 2•• | 20x + 20y | 4 + 12 | 65.0 | 14.5 | 53 | 46.7 |
| BGE 18254 | Sierra Nevada | Granada | 1 | 7 | 2 + 12 | 64.5 | 14.7 | 42 | 38.7 |
| BGE 18355 | Ruso | Ávila | Null | 7 + 8 | 2 + 12 | 78.5 | 12.5 | 60 | 35.7 |
| BGE 18671 | Blat Mort | Baleares | 2* | 20x + 20y | 2 + 12 | 48.5 | 15.4 | 60 | 52.3 |
| BGE 18922 | Raspinegro | Madrid | 2* | 20x + 20y | 4 + 12 | 30.0 | 13.0 | 33 | 40.3 |
| BGE 19317 | Gejar | — | 1 | 20x + 20y | 2 + 12 | 21.0 | 14.2 | 28 | 56.7 |
| BGE 19324 | Villaverde de Trucios | Cantabria | 2* | 20x + 20y | 2 + 12 | 36.5 | 13.7 | 36 | 56.6 |
| BGE 19329 | Blando Granja Melilla | — | 2* | 20x + 20y | 2 + 12 | 38.0 | 12.9 | 47 | 39.8 |
| BGE 38604 | Trigo Medina | Badajoz | 2* | 20x + 20y | 4 + 12 | 75.0 | 15.1 | 60 | 34.5 |